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Albophoma belongs in *Ophiocordycipitaceae*, with a new record from China

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Abstract—A fungus isolated from forest soil in Guizhou Province, China, was identified by morphology and phylogenetic analyses as *Albophoma yamanashiensis*, a new record from China. The combined ITS+LSU phylogenetic tree places *Albophoma* (previously a conidial coelomycetous genus of unknown affinity) in *Ophiocordycipitaceae* and sister to *Tolypocladium*.

Key words—family classification, *Hypocreales, Sordariomycetes,* taxonomy

Introduction

Albophoma was established as a monotypic genus by Kobayashi & al. (1994), typified by *A. yamanashiensis* isolated from forest soil in Yamanashi Prefecture, Japan. *Albophoma* has been classified as a conidial coelomycetous genus of unknown affinity, characterized by pure white fleshy pycnidia and small globular conidia with many frills on their wall surfaces and a thick basal scar. No additional *Albophoma* species have been proposed (http://www.mycobank.org).

During a survey of soil coelomcycete diversity in southwest China's Guizhou Province, we isolated two fungal strains that showed the

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Table 1. Sequences used in phylogenetic analyses.

Species	Strain	GenBank accession no.	
		ITS	LSU
Cordyceps militaris	OSC 93623	JN049825	AY184966
C. kyusyuensis	EFCC 5886	—	EF468813
Polycephalomyces formosus	ARSEF1424	KF049661	KF049634
P. ramosus	MFLU 18-0162 T	MK863250	MK863050
Purpureocillium lilacinum	CBS 284.36T	AY624189	—
	CBS 431.87	AY624188	EF468844
P. takamizusanense	NHJ 3497	—	EU369033
Perennicordyceps prolifica	NBRC 101750	JN943340	JN941433
	NBRC 103838	JN943339	JN941434
P. cuboidea	NBRC 100941	AB378666	AB378646
	NBRC 101742	AB378667	AB378648
P. paracuboidea	NBRC 101742	—	KF049630
P. ryogamiensis	NBRC 103837	—	JN941439
Tolypocladium capitatum	NBRC 106327	JN943317	JN941404
T. inflatum	CBS 567.84 T	MH861779	MH873477
T. japonicum	OSC 110991	JN049824	DQ518761
T. ophioglossoides	NBRC 106331	JN943320	JN941408
Ophiocordyceps melolonthae	OSC 110993	—	DQ518762
	Ophgrc679	—	KC610768
O. variabilis	ARSEF 5365	—	DQ518769
	OSC 111003	—	EF468839
Hirsutella fusiformis	ARSEF 5474	—	KM652110
H. liboensis	ARSEF 9603 T	KM652163	KM652115
H. lecaniicola	ARSEF 8888	KM652162	KM652114
H. illustris	ARSEF 5539	KM652160	KM652112
Paraisaria orthopterorum	TBRC 9710 T	MH754743	MK332582
P. phuwiangensis	BBH 43491 T	MH188542	MK192058
P. rosea	HKAS 102546	MN947222	MN943842
P. heteropoda	EFCC 10125	JN049852	EF468812
P. coenomyiae	NBRC 106964	AB968397	AB968413
Albophoma yamanashiensis	JCM 11844 T	LC228661	LC228718
	HGUP 20-8	**OK559516**	**OK562790**
	HGUP 20-50	**OK559517**	**OK562791**

Type strains are marked by T. New sequences are set in **bold** font.

morphological characters of *A. yamanashiensis* on potato dextrose agar (PDA). Based on morphological and ITS and LSU sequence analyses, we found the two strains were conspecific and identified them as *A. yamanashiensis*, a new record from China. The phylogeny supports *Albophoma* in *Ophiocordycipitaceae* G.H. Sung & al. (Sung & al. 2007), as a sister genus to *Tolypocladium* W. Gams.

Materials & methods

Samples, fungal isolates, morphological observations

Soil samples were collected in 2019 from Fodingshan National Nature Reserve in Shiqian County, Guizhou Province, China, and a plate dilution method was used to isolate soil fungi (Turmel & al. 2010). The conidiophores and conidia were morphologically examined and photographed at 400× and 1000× with a Nikon 90i microscope. The specimens were conserved in the Herbarium of the Department of Plant Pathology, Agricultural College, Guizhou University, China (HGUP).

DNA extraction, amplification, sequencing

Genomic DNA was extracted from the fresh mycelia grown on PDA, using the Biomiga Fungal gDNA Kit GD2416 following the manufacturer's instructions. The primers ITS4/ITS5 (White & al. 1990) were used for the ITS region amplification, and LR0R/LR5 were used to amplify a segment of the large subunit rDNA (Vilgalys & Hester 1990). The purified PCR products were sequenced by Sangon Biotech Co., Ltd. Sequences were downloaded from GenBank for phylogenetic analyses, and the newly generated sequences were deposited in GenBank (Table 1).

Sequence analyses

The newly generated sequences were compared with related sequences by BLAST searches. Sequences were aligned using MAFFT v.6 (Katoh & Toh 2010) and edited manually using MEGA 6.0. The combined ITS and LSU rDNA sequences were analyzed phylogenetically using Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI). MP analysis was performed using PAUP* 4.0b10 (Swofford 2003). ML analysis was conducted using RAxML-HPC BlackBox v.8.2.12 (Stamatakis 2014) via the CIPRES Science Gateway platform (Miller & al. 2010) with 1000 bootstrap replicates. The model for BI analysis was determined using jModelTest 2.0 (Posada 2008), and TIM2+I+G was estimated as the best-fit model under the output strategy of Akaike Information Criterion (AIC). BI analysis was performed via MrBayes v3.2.7 (Ronquist & al. 2012). The phylogenetic tree was viewed in FigTree v1.4.4 and edited in Adobe Illustrator CS5 (AI).

Taxonomy

Albophoma yamanashiensis Tak. Kobay., Masuma, Ōmura & Kyoto Watan., Mycoscience 35 (4): 399 (1994) Fig. 1

Colonies on PDA flat, white, attaining 20 mm diam. after 3 weeks at 28°C, with numerous pycnidia on the surface. Mycelium superficial, hyphae hyaline, septate, smooth, branched, 2–3.7 µm wide. Conidiophores arising from the inner hyphae of pycnidium, producing conidia holoblastically. Pycnidia white, globular or somewhat oblate, 120–185 µm in diam. Conidia one-celled, hyaline, globular, 0.7–2.0 µm in diam.

Specimens examined: **CHINA, Guizhou Province, Shiqian County**, Fodingshan National Nature Reserve, 1164 m asl, from a forest soil sample, 17 October 2019, W.Y. Jia (HGUP 20–8; GenBank OK559516, OK562790); (HGUP 20–50; GenBank OK559517, OK562791).

Phylogeny

The ML phylogenetic tree is shown (Fig. 2), with *Cordyceps militaris* and *C. kyusyuensis* as outgroup. The 31 ingroup sequences grouped within *Ophiocordycipitaceae*, which formed two large branches with full bootstrap support (ML/MP/BI = 100%/100%/1). The sequences generated from our two strains, HGUP 20–8 and HGUP 20–50 clustered together with an ex-type strain of *A. yamanashiensis* in a fully supported clade (ML/MP/BI = 100%/100%/1), and was sister to the *Tolypocladium* group (ML/MP/BI = -/60%/1). Therefore, phylogenetic analyses based on two gene loci support the identification of the two strains as *A. yamanashiensis* and identify *Albophoma* as belonging in *Ophiocordycipitaceae*.

Discussion

Our two Chinese strains identified as *A. yamanashiensis* are morphologically very similar to the fungus described by Kobayashi & al. (1994), and their ITS and LSU sequences confirmed this identification. Additionally, although *Albophoma* is phylogenetically close to *Tolypocladium* in the phylogram of *Ophiocordycipitaceae* (Fig. 2), there are obvious morphological differences separating the two genera. *Tolypocladium* is easily distinguished by its usually swollen phialides that give rise to conidia enteroblastically, and it produces no fleshy, white pycnidia (Gams 1971, Wang & al. 2022). Both morphological and phylogenetic analyses place our strains HGUP 20–8 and HGUP 20–50 as *A. yamanashiensis* within *Ophiocordycipitaceae*.

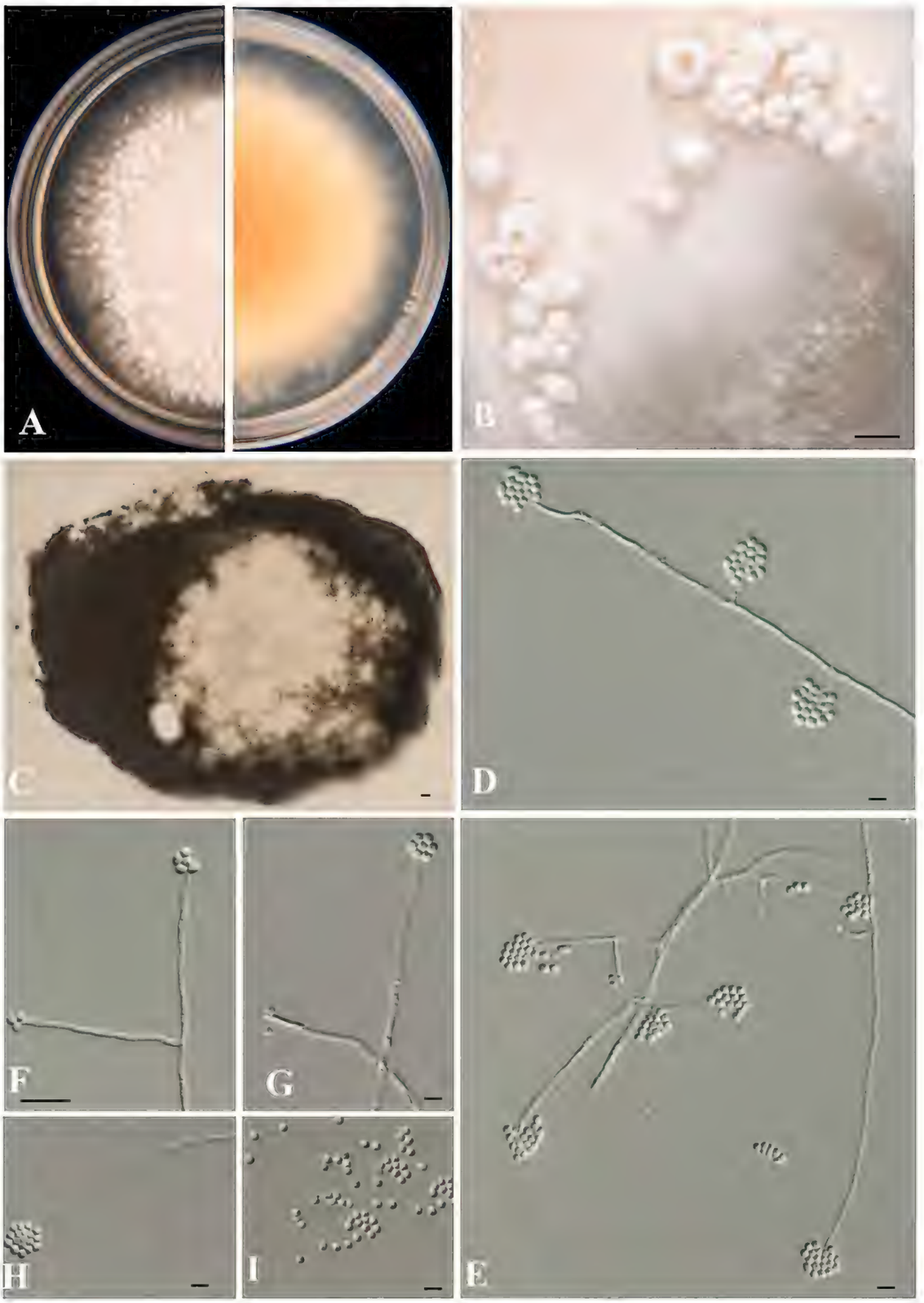

Fig. 1. *Albophoma yamanashiensis* (HGUP 20–50). A: Colony on PDA for three weeks producing many pycnidia; B: White, globular pycnidia; C: Cross section of pycnidium having numerous minute conidia; D–I: Acremonium-type conidia. Scale bars: B = 1 mm; C = 100 µm; D–I = 50 µm.

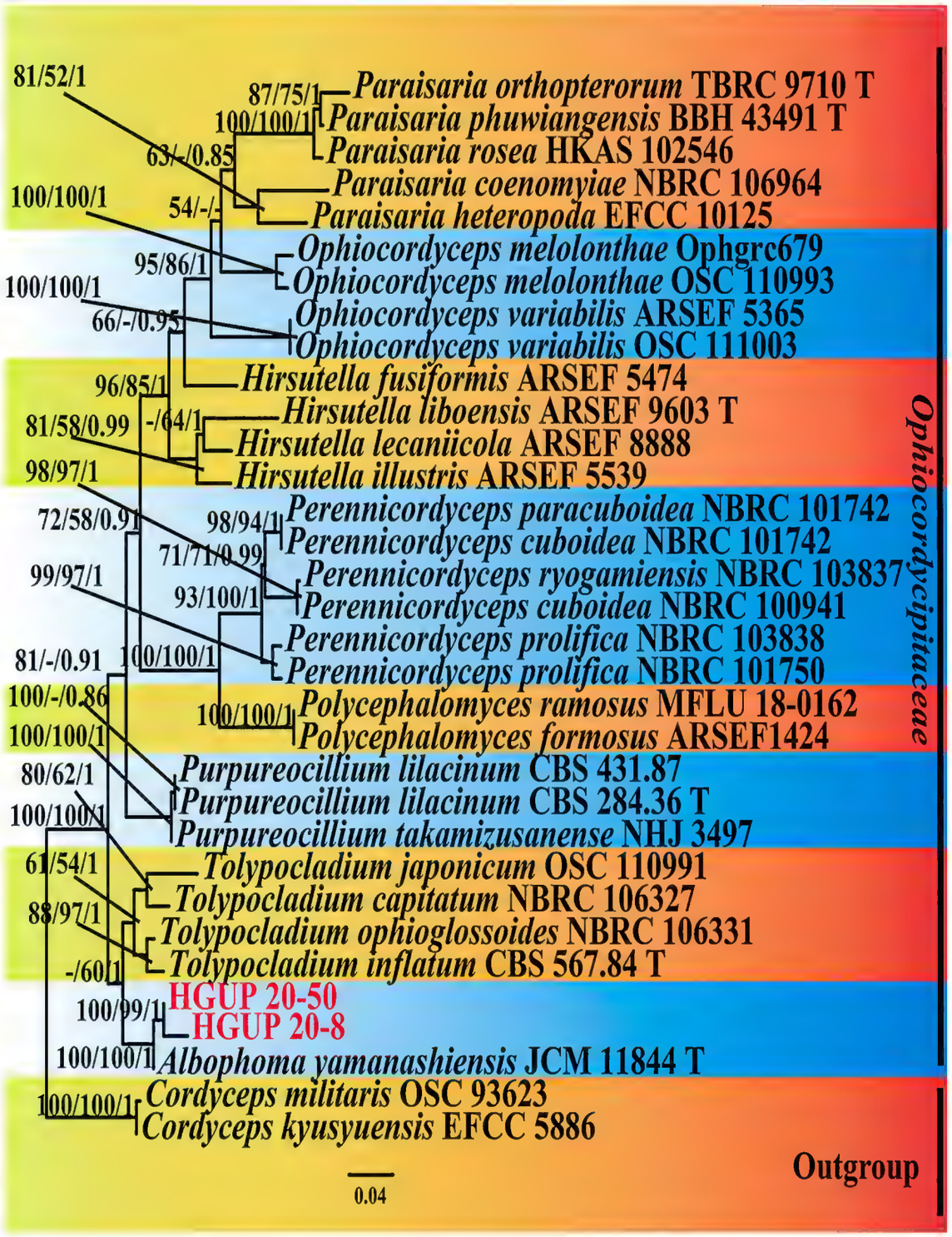

Fig. 2. The system diagram generated by Maximum Likelihood analysis of the combined LSU and ITS sequence data. Branch support displayed at the nodes as: Maximum Likelihood and Maximum Parsimony bootstrap support values >50% / Bayesian posterior probabilities ≥0.85. *Cordyceps militaris* and *C. kyusyuensis* are used as outgroup. Our strains are set in red font, and ex-type strains are marked by T.

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